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TECHNICAL NOTE

Isolation and characterization of twelve microsatellite loci for the Japanese Devilray *(Mobula japanica)*

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Abstract Twelve polymorphic microsatellites loci were characterized for *Mobula japanica* (Japanese Devilray) using an enrichment protocol. All but two loci were in Hardy–Weinberg equilibrium with no evidence of linkage disequilibrium or null-alleles for a sample of 40 individuals from two populations. The number of alleles varied from 5 to 28. Expected heterozygosity ranged from 0.2332 to 0.9589, making these microsatellite loci good candidates for population genetic studies.

Keywords Elasmobranch · *Mobula japanica* · Microsatellite · Population genetics · Polymorphism

The Japanese Devilray *(Mobula japanica)* is believed to have a circumglobal distribution throughout all temperate and tropical seas, although genetic analyses may identify separate populations or even cryptic species over such a wide range (Notarbartolo-di-Sciara 1987). The species reaches a disc width (DW, measured from wingtip to wingtip) of 310 cm. It is mainly pelagic and found inshore, offshore and, possibly, in oceanic environments (Last and Stevens 1994). *M. japanica* is listed as "Near Threatened" by the International Union for Conservation of Nature (IUCN: www.iucn.org/redlist), due to high (by) catch rates, increasing demand and low reproductive potential. Therefore, data regarding current genetic structure and migration patterns are needed to design effective conservation strategies (Graves 1998). Species-specific microsatellite markers provide a means of obtaining these data for threatened and endangered taxa. Here we report on the isolation and characterization of 12 novel microsatellite loci in *M. japanica*.

Genomic libraries enriched for microsatellite motifs were constructed by Genetic Identification Services (GIS, http://www.genetic-id-services.com; Chatsworth, CA, USA). Libraries were built using a sample containing 100 μg of genomic DNA extracted from tail tissue of a single individual *M. japanica* collected in El Pardito, Baja California Sur, Mexico. The sample was stored in 90% ethanol and extracted using a Qaigen Blood and Tissue DNA purification kit. Libraries were enriched for CA, CATC, TACA, TAGA motifs. GIS sequenced 54 microsatellite-containing clones using universal M13 primers, and designed primers using DesignerPCR version 1.03 (Research Genetics, Inc.).

We tested these 54 microsatellites, using a dye-labeled universal primer system (Schuelke 2000) with an M13 tagged tail (5′-CAC GAC GTT GTA AAA CGA C-3′) added to the 5′ end of the forward primer. Amplification reactions were carried out in a single nested reaction on an Applied Biosystems GeneAmp PCR 9700 in a total volume of 12 μL containing 1× PCR Mastermix (2.5 mM TAPS pH9.5, 5.0 mM KCl, 0.2 mM $MgCl_2$, 20.0 μM of each dNTP, Taq 0.5u/μL, Thermo Scientific), 2 pmols of the M13 labeled forward primer, 9 pmol of the reverse primer,

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9 pmols of the fluorescently-labeled M13 primer (Fluo, Tamra, Hex; Sigma-Genosys) and approximately 2 ng of DNA template. The following PCR temperature profile was used: 5 min at 94°C, followed by 10 cycles of 30 s at 94°C, 45 s at the primer specific T_a, 45 s at 72°C, followed by 20 cycles of 30 s at 94°C, 45 s at ((primer specific T_a) minus 2°C), 45 s at 72°C and a final extension of 72°C for 10 min. Microsatellite amplifications were mixed with Applied Biosystems GeneScan 500 Rox size standard and then run on an ABI 3100 automated sequencer, and scored using the software GENEMAPPER3.7 (Applied Biosystems). Twelve out of the original 54 loci produced successful PCR amplification. Locus-specific dye-labeled primers (6FAM, NED, PET, VIC: Applied Biosystems) were used for those 12 loci.

Allelic diversity and heterozygosity were estimated using 40 individuals from two populations (20 from Puerto Lopez, Ecuador and 20 from La Paz, Baja California, Mexico). All 12 microsatellites were amplified in two independent multiplex reactions (Applied Biosystems GeneAmp PCR 9700; Panel 1 or 2, Table 1). The PCR reaction volume of 10 μL contained 5 μL Multiplex-PCR Master Mix (QIAGEN), 1 μM Q-solution (QIAGEN), 2 μM of each primer (Table 1) and 0.5 μL template DNA.

Table 1 Characterization of twelve polymorphic microsatellite loci in *Mobula japanica*

Locus	Genbank acc. no.	Primer label	Panel	Primer sequence (5′–3′)	Repeat motif	Amp. range	N_a	H_o/H_e
MOJA2	JF800912	6FAM	2	F: AGGAATGCTCCAAATAAGA	(CA)8 TACGC (CA)4 CG (CA)5 CG (CA)4 (CG)2 (CA)5 (CG)2 (CA)4	178–332	25	0.9250
				R:ACGTCTTCATAGCAGCAGTA				0.9434
MOJA4	JF800913	PET	1	F:CAATGTCACTTTTAGCACACT	(CA)3 AA (CA)30 CCT (CA)2	304–356	28	0.9750
				R:AATTCAGCGTGAGTAAACTC				0.9589
MOJA10	JF800914	6FAM	2	F:GGTCTTGTTTCTGAAGTCCAGT	(CA)15	114–148	20	0.9250
				R:TGCCGATTACTAAAGAATGACA				0.9244
MOJA112	JF800915	VIC	1	F:CTGGCTGTTCTCTTTCCCAC	(GT)3 TTG (GT)14 TTATTGTGCGTATTT (GT)3 TTA (GT)4 GCTAAT (TC)2 CATTTTG (GT)3	218–234	9	0.5750
				R:CTCCCTTCAGACCTGGACTG				0.7604
MOJA124	JF800916	PET	2	F:GCAAAAAAAGACACTGAACTGA	(CA)10	124–140	8	0.8000
				R:GACCTGAAGCATCAACTGTTTA				0.7756
MOJA133	JF800917	VIC	2	F:TCCCGTAAACACTCACAGG	(CA)4 TG (CA)13	208–226	5	0.7250
				R:ATTTCTTCCCCATTCTGATG				0.6994
MOJA134	JF800918	VIC	1	F:CCTTTACGCACACATACAAAC	(CA)3 TTCATTCAAAA (CA)2 TACATA (CA)2 CGTA (CA)2 GATATC (CA)2 GGCATAGTCATGTATA (CA)23	148–186	20	**0.9250**
				R:CACCATCAACCCTTTCTAAGA				0.9348
MOJC7	JF800919	PET	1	F:AAGCCCTGGTGTGTGTCTG	(GTAT)4 AT (GTAT)3	128–156	6	0.2500
				R:TTTGGTAATGAAATGGAACTGG				0.2332
MOJD9	JF800920	6FAM	1	F:TGCTTTGAGACTGGTTTGC	(CT)5 (ATCT)4 CT (ATCT)3 AC (CT)3 ATCTGTCTATCTT (CT)3 CCTT (CT)2	120–144	7	0.4750
				R:TGGGAACTTTTACTGAGAGGG				0.4994
MOJD10	JF800921	VIC	2	F:ACTTATTTCCATCCGGCATAGT	(TATC)6	236–272	8	0.7000
				R:TCCAGGATATAAAGCGCAGTAG				0.5972
MOJD104	JF800922	NED	2	F:TGGCACATAATGATGATGATG	(TAGA)9	256–280	10	**0.8500**
				R:AGGATGGTAGAGGAACTCAGTG				0.8548
MOJD112	JF800923	PET	2	F:AAAATGCAGCCAGAACATG	(TAGA)7 TTGACAGA (TAGA)5 CAGA (TAGA)2 (CAGA)2 TAGACAGA (TAGA)2 CAAATAGACAGATAGATAGG (TAGA)3 TTGA (CAGA)2 TAGATAAA (CAGA)2 TAGA (CAGA)2 TAGACAGA (TAGA)2 CAAATAGACAGATAGATAGG (TAGA)2	148–400	21	0.9750
				R:CGCACTTGTAATGCTACTGTG				0.8791

GenBank accession numbers, primer label, amplification multiplex panel, forward (F) and reverse (R) primer sequences, repeat motif and amplification range are given for each locus. N_a, H_O and H_E represent number of alleles, observed heterozygosity and expected heterozygosity identified from 40 assayed individuals. Observed heterozygosity numbers in bold show significant deviations from HWE ($P < 0.05$)

The following PCR temperature profile was used: 15 min at 95°C, followed by 35 cycles of 30 s at 94°C, 90 s at 57°C, 60 s at 72°C and a final extension of 72°C for 10 min. Diluted microsatellite amplifications (1:10) were mixed with Applied Biosystems GeneScan 500 LIZ size standard and then run on an ABI 3100 automated sequencer, and scored using the software GENEMAPPER3.7 (Applied Biosystems). Tests for zygotic (Hardy–Weinberg) equilibrium and gametic disequilibrium were conducted in Arlequin version 3.5 (Excoffier et al. 2005). A search for null alleles was conducted using Microchecker version 2.2.3 (van Oosterhout et al. 2006). We observed 5–28 alleles per locus (Table 1), with an average of 14 alleles per locus. Expected heterozygosity values ranged from 0.2332 to 0.9589 (Table 1). All loci except two (A134 and D104) were in Hardy–Weinberg equilibrium (HWE). An exact test for linkage disequilibrium between loci within the populations showed no locus pairs with significant *P*-values after Bonferroni correction. There was no evidence for null-alleles as judged empirically or from Microchecker (van Oosterhout et al. 2006).

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